Published in final edited form as:

J Med Chem. 2012 September 13; 55(17): 7342–7345. doi:10.1021/jm3011977.

Scaling the Druggability Landscape of Human Bromodomains, a New Class of Drug Targets

Guangtao Zhang, Roberto Sanchez, and Ming-Ming Zhou*

From Department of Structural and Chemical Biology, Mount Sinai School of Medicine, New York, NY 10029, USA

Gene transcriptional activation of the human genome in response to physiological and environmental stimuli requires chromatin structure changes defined by enzymes that modify chromatin and directed by proteins that interact with chromatin in a modification-sensitive manner. This highly complex system operates with a large number of chemical modifications on chromatin (both DNA and histones) and transcription-associated proteins.¹ Of these, lysine acetylation functions to facilitate chromatin opening and productive transcriptional machinery assembly required for gene activation. These activities are directed by the acetyl-lysine binding activity of the bromodomain (BrD), a fundamental molecular mechanism for gene transcriptional activation that was discovered in the structural biology study of the histone acetyltransferase (HAT) transcriptional co-activator PCAF.² The human genome encodes a total of 61 bromodomains in 46 chromatin regulator proteins, some of which comprise multiple bromodomains.² As a key epigenome reader, the bromodomain is almost solely responsible for binding to acetylated-lysine in histones and transcription-associated proteins, thereby orchestrating gene transcription in chromatin in an ordered fashion.² Recent studies show that pharmacological small molecule modulation of the acetyl-lysine binding activity of BrD proteins such as the BET (Bromodomain and Extra-Terminal domain) family protein BRD4 and the HAT co-activator CBP/p300 dictates gene transcription outcome in disease models³ such as multiple myeloma, lymphoma, acute myeloid leukemia, mixed lineage leukemia, HIV-associated kidney disease, and ischemia, indicating these bromodomains as attractive drug targets for diseases including cancer and inflammation.

All bromodomains share an evolutionarily conserved structural fold consisting of a left-handed four-helix bundle with two inter-helix-connecting loops, termed ZA and BC loops, which together constitute the acetyl-lysine binding pocket.² Given high degree variations in the amino acid sequence and structural flexibility of the ZA and BC loops, the acetyl-lysine binding pocket poses a challenge to developing potent and selective small molecule inhibitors for bromodomains. In an effort to explore the physicochemical basis for small molecule inhibition of bromodomains, Vidler *et al* conducted a family-wide survey of the druggability landscape of the acetyl-lysine binding site in human bromodomains.⁴

Generally speaking, a target protein is considered druggable if it can be modulated *in vivo* by a drug-like molecule.⁵ Structure-based target druggability assessment, however, uses a less restrictive definition of druggability, namely the ability of a protein to bind to drug-like molecules with high affinity. While related, the two definitions are not identical. A high-affinity drug-like ligand may not be active *in vivo* for a number of reasons including

To whom correspondence should be addressed to: Ming-Ming Zhou, 1425 Madison Avenue, Box 1677, New York, NY 10029. Fax: 212-849-2456; ming-ming.zhou@mssm.edu.

bioavailability. Structure-based druggability assessment predicts a degree by which a ligand-binding site in a protein is able to bind to a drug-like molecule with high affinity.⁵

The recent availability of several validation sets of druggable *versus* undruggable targets has led to the development of a number of computational methods.⁵ Most methods combine geometrical and physicochemical properties of the protein surface to define mainly the size, shape, and hydrophobicity of binding pockets, although other properties may also be included.⁵ Among these SiteMap is one of the most widely used,⁵ and is also the one used in the Vidler study of bromodomains. SiteMap combines binding site identification with druggability assessment. First, a binding site is identified as a set of reasonably enclosed points that are outside the protein; groups of these points define 'sites' that are characterized their size, degree of enclosure by the protein, hydrophilicity, hydrophobicity, and other properties. A SiteScore is defined using a subset of these properties and used for binding-site identification. A different score, Dscore, which uses the same properties as the SiteScore but with different coefficients, is then used to assess the druggability of the predicted binding sites. Hydrophobicity plays a larger role in Dscore than in SiteScore due to the fact that "undruggable" sites typically are much more hydrophilic and much less hydrophobic than "druggable" sites.

Using SiteMap, Vidler and coworkers assessed the druggability of human bromodomains using 105 crystal structure entries available in Protein Data Bank, which encompass 33 of the 61 human bromodomains. Eight residues near the acetyl-lysine binding site of the BET protein BRD4 (i.e. W81, L92, L94, N140, D144, D145, I146, and M149) were utilized as references (Figure 1A,B). In addition to the key residues that interact with the bound acetyl-lysine, five water molecules are found at the conserved sites within the ligand-binding pocket in many different bromodomains, suggesting that they are an integrated part of the acetyl-lysine binding pocket (Figure 1C). As such, these bound water molecules were included in the SiteMap analysis of protein druggability. Superimpositions of these structures lead to classification of the human bromodomain family into 9 groups that show distinctive structural features. The Dscore analysis further predicted that the BET bromodomains, Group 1, are highly druggable, which is in an agreement with the fact that several highly potent BET bromodomain-specific small molecule inhibitors such as JQ1, MS417 and iBET have been reported in the literature.^{3, 6} Group 2, which consists of the bromodomains from transcriptional cofactor proteins GCN5, PCAF, FALZ, and CECR2, show a highly predicted druggability. Low micromolar affinity inhibitors for this group of bromodomains have also been reported,⁶ which represent attractive drug targets for future drug design efforts. Note that although the CBP/p300 bromodomain shows marked variations in structural features as compared to the other groups, it has intermediate druggability. This prediction is supported by the recent development of the CBP bromodomain inhibitors using target structure-guided methods.^{3,6} The prediction further reveals that other groups of bromodomains appear less druggable than the BET family bromodomains. These groups and their structural correlations were depicted in a bromodomain phylogenetic tree.

One of the limitations of SiteMap, and most druggability assessment methods, is that the druggability measure corresponds to the predicted binding site, which may or may not be an accurate representation of the true binding site for a ligand. For instance, it is possible that a ligand will exploit multiple neighboring sites that are considered independent by SiteMap, which might results in greatly increased druggability.

While this study represents the first family-wide druggability analysis for bromodomains, some predictions shall be interpreted with caution. First, as reported previously, the inter-helical ZA and BC loops of bromodomains are highly flexible.² Local conformational

changes in these loops upon binding to a small-molecule ligand could result in changes in the pocket volume and enclosure, leading to a different Dscore value. Consequently, new predictions of druggability score could vary as more available bromodomain structures are included in calculations. Second, possible contribution of secondary cavities adjacent to the acetyl-lysine binding site was not fully explored in this study. Such neighboring cavities could provide new opportunities to improve affinity for small molecules. Lastly, as more druggability prediction programs such as Focket, and MAP_{POD}⁵ become capable of handling bound water molecules, it will be interesting to compare results obtained with different computational methods. This will enable one to validate and even further improve the fidelity of such predictions.

As a distinct class of protein-protein interaction (PPI) domains that function to regulate gene transcription, bromodomains contrast with the classical highly-druggable drug targets such as protein kinases, GPCRs, and proteases⁷. In GPCRs the ligand-binding pockets generally are deeply buried in the protein and often nearly fully enclosed, which gives them more contacts with the protein for the same pocket volume⁷. In kinases the pockets are large and very deep (ATP-binding site)⁷, thereby providing many contacts with the protein for the size of the ligand. In comparison, the acetyl-lysine binding pockets in bromodomains are not as large, deep, and enclosed as those of these highly-druggable targets. As such, bromodomains probably lie in between these highly-druggable targets and difficult targets such as protein-protein interaction surfaces. Nevertheless, it is difficult to perform a quantitative comparison due to the fact that druggability measures are merely trying to classify targets as "druggable", "difficult", and "undruggable", rather than correctly rank targets within one category.

It is worth noting that what one considers druggable now is biased by the type of molecules that have been used as starting points for drug discovery⁷. Discovery of new types of molecules may lead to redefinition of what is druggable. For example it has been shown that one can define libraries of small molecules that have a higher chance of inhibiting PPIs.⁷ For this reason, it is important to emphasize that eventually one needs to attempt the targeting of all medically relevant bromodomains (and targets in general), not just those that are currently considered druggable. Accordingly, the classification of druggability of bromodomains is useful as it predicts which ones could be targeted using current approaches (i.e. low hanging fruit), and which ones would require new developments before one could successfully target them (e.g. different small-molecule libraries such as the case for PPIs). They certainly should not be simply abandoned because they seem intractable in the light of current druggability estimates.

As a parallel in drug discovery that is moving from highly druggable targets such as kinases and GPCRs to more challenging PPI targets, rapidly growing ligand design efforts for bromodomains are expected to progress from targeting a few druggable bromodomains to more difficult ones. The outcome of these studies will undoubtedly enable us to validate members of this new class of drug targets and develop more effective targeted epigenetic therapies for human diseases including cancer and inflammation.

Acknowledgments

The work was in part supported by the grants from the National Institutes of Health (to M.-M.Z.).

References

1. Kouzarides T. Chromatin modifications and their function. *Cell*. 2007; 128:693–705. [PubMed: 17320507]

2. (a) Dhalluin C, Carlson JE, Zeng L, He C, Aggarwal AK, Zhou MM. Structure and ligand of a histone acetyltransferase bromodomain. *Nature*. 1999; 399:491–496. [PubMed: 10365964] (b) Filippakopoulos P, Picaud S, Mangos M, Keates T, Lambert JP, Barsyte-Lovejoy D, Felletar I, Volkmer R, Muller S, Pawson T, Gingras AC, Arrowsmith CH, Knapp S. Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell*. 2012; 149:214–231. [PubMed: 22464331] (c) Jacobson RH, Ladurner AG, King DS, Tjian R. Structure and function of a human TAFII250 double bromodomain module. *Science*. 2000; 288:1422–1425. [PubMed: 10827952] (d) Owen DJ, Ornaghi P, Yang JC, Lowe N, Evans PR, Ballario P, Neuhaus D, Filetici P, Travers AA. The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase Gcn5p. *EMBO J*. 2000; 19:6141–6149. [PubMed: 11080160] (e) Sanchez R, Zhou MM. The role of human bromodomains in chromatin biology and gene transcription. *Curr Opin Drug Discov Devel*. 2009; 12:659–665. (f) Zeng L, Zhou MM. Bromodomain: an acetyl-lysine binding domain. *FEBS Lett*. 2002; 513:124–128. [PubMed: 11911891]
3. (a) Borah JC, Mujtaba S, Karakikes I, Zeng L, Muller M, Patel J, Moshkina N, Morohashi K, Zhang W, Gerona-Navarro G, Hajjar RJ, Zhou MM. A small molecule binding to the coactivator CREB-binding protein blocks apoptosis in cardiomyocytes. *Chem Biol*. 2011; 18:531–541. [PubMed: 21513889] (b) Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, Robson SC, Chung CW, Hopf C, Savitski MM, Huthmacher C, Gudgin E, Lugo D, Beinke S, Chapman TD, Roberts EJ, Soden PE, Auger KR, Mirguet O, Doehner K, Delwel R, Burnett AK, Jeffrey P, Drewes G, Lee K, Huntly BJ, Kouzarides T. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature*. 2011; 478:529–533. [PubMed: 21964340] (c) Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastitis E, Gilpatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, Anderson KC, Kung AL, Bradner JE, Mitsiades CS. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell*. 2011; 146:904–917. [PubMed: 21889194] (d) Mertz JA, Conery AR, Bryant BM, Sandy P, Balasubramanian S, Mele DA, Bergeron L, Sims RJ. Targeting MYC dependence in cancer by inhibiting BET bromodomains. *P Natl Acad Sci USA*. 2011; 108:16669–16674. (e) Zhang G, Liu R, Zhong Y, Plotnikov AN, Zhang W, Zeng L, Rusinova E, Gerona-Navarro G, Moshkina N, Joshua J, Chuang PY, Ohlmeyer M, He JC, Zhou MM. Down-regulation of NF-kappaB transcriptional activity in HIV-associated kidney disease by BRD4 inhibition. *J Biol Chem*. 2012; 287:28840–28851. [PubMed: 22645123] (f) Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature*. 2011; 478:524–528. [PubMed: 21814200]
4. Vidler LR, Brown N, Knapp S, Hoelder S. Druggability Analysis and Structural Classification of Bromodomain Acetyl-lysine Binding Sites. *J Med Chem*. 2012
5. (a) Cheng AC, Coleman RG, Smyth KT, Cao Q, Soulard P, Caffrey DR, Salzberg AC, Huang ES. Structure-based maximal affinity model predicts small-molecule druggability. *Nat Biotechnol*. 2007; 25:71–75. [PubMed: 17211405] (b) Hajduk PJ, Huth JR, Tse C. Predicting protein druggability. *Drug Discov Today*. 2005; 10:1675–1682. [PubMed: 16376828] (c) Halgren TA. Identifying and characterizing binding sites and assessing druggability. *J Chem Inf Model*. 2009; 49:377–389. [PubMed: 19434839] (d) Krasowski A, Muthas D, Sarkar A, Schmitt S, Brenk R. DrugPred: A Structure-Based Approach To Predict Protein Druggability Developed Using an Extensive Nonredundant Data Set. *J Chem Inf Model*. 2011; 51:2829–2842. [PubMed: 21995295] (e) Le Guilloux V, Schmidtke P, Tuffery P. Fpocket: an open source platform for ligand pocket detection. *BMC Bioinformatics*. 2009; 10:168. [PubMed: 19486540] (f) Nisius B, Sha F, Gohlke H. Structure-based computational analysis of protein binding sites for function and druggability prediction. *J Biotechnol*. 2012; 159:123–134. [PubMed: 22197384] (g) Schmidtke P, Barril X. Understanding and Predicting Druggability. A High-Throughput Method for Detection of Drug Binding Sites. *J Med Chem*. 2010; 53:5858–5867. [PubMed: 20684613] (h) Seco J, Luque FJ, Barril X. Binding Site Detection and Druggability Index from First Principles. *J Med Chem*. 2009; 52:2363–2371. [PubMed: 19296650] (i) Sheridan RP, Maiorov VN, Holloway MK, Cornell WD, Gao YD. Drug-like Density: A Method of Quantifying the "Bindability" of a Protein Target Based on a Very Large Set of Pockets and Drug-like Ligands from the Protein Data Bank. *J Chem Inf Model*. 2010; 50:2029–2040. [PubMed: 20977231] (j) Sugaya N, Ikeda K. Assessing the

- druggability of protein-protein interactions by a supervised machine-learning method. *BMC Bioinformatics*. 2009; 10:263. [PubMed: 19703312] (k) Volkamer A, Kuhn D, Grombacher T, Rippmann F, Rarey M. Combining Global and Local Measures for Structure-Based Druggability Predictions. *J Chem Inf Model*. 2012; 52:360–372. [PubMed: 22148551]
6. (a) Dekker FJ, Ghizzoni M, van der Meer N, Wisastra R, Haisma HJ. Inhibition of the PCAF histone acetyl transferase and cell proliferation by isothiazolones. *Bioorg Med Chem*. 2009; 17:460–466. [PubMed: 19111471] (b) Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE. Selective inhibition of BET bromodomains. *Nature*. 2010; 468:1067–1073. [PubMed: 20871596] (c) Hewings DS, Wang M, Philpott M, Fedorov O, Uttarkar S, Filippakopoulos P, Picaud S, Vuppasetty C, Marsden B, Knapp S, Conway SJ, Heightman TD. 3,5-dimethylisoxazoles act as acetyl-lysine-mimetic bromodomain ligands. *J Med Chem*. 2011; 54:6761–6770. [PubMed: 21851057] (d) Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, Chandwani R, Marazzi I, Wilson P, Coste H, White J, Kirilovsky J, Rice CM, Lora JM, Prinjha RK, Lee K, Tarakhovsky A. Suppression of inflammation by a synthetic histone mimic. *Nature*. 2010; 468:1119–1123. [PubMed: 21068722] (e) Sachchidanand, Resnick-Silverman L, Yan S, Mutjaba S, Liu WJ, Zeng L, Manfredi JJ, Zhou MM. Target structure-based discovery of small molecules that block human p53 and CREB binding protein association. *Chem Biol*. 2006; 13:81–90. [PubMed: 16426974] (f) Zeng L, Li J, Muller M, Yan S, Mujtaba S, Pan C, Wang Z, Zhou MM. Selective small molecules blocking HIV-1 Tat and coactivator PCAF association. *J Am Chem Soc*. 2005; 127:2376–2377. [PubMed: 15724976]
7. (a) Billingsley ML. Druggable Targets and Targeted Drugs: Enhancing the Development of New Therapeutics. *Pharmacology*. 2008; 82:239–244. [PubMed: 18802381] (b) Hopkins AL, Groom CR. The druggable genome. *Nat Rev Drug Discov*. 2002; 1:727–730. [PubMed: 12209152] (c) Mason JS, Bortolato A, Congreve M, Marshall FH. New insights from structural biology into the druggability of G protein-coupled receptors. *Trends Pharmacol Sci*. 2012; 33:249–260. [PubMed: 22465153] (d) Metz A, Ciglia E, Gohlke H. Modulating Protein-Protein Interactions: From Structural Determinants of Binding to Druggability Prediction to Application. *Curr Pharm Des*. 2012 (e) Reynes C, Host H, Camproux AC, Laconde G, Leroux F, Mazars A, Deprez B, Fahraeus R, Villoutreix BO, Sperandio O. Designing Focused Chemical Libraries Enriched in Protein-Protein Interaction Inhibitors using Machine-Learning Methods. *Plos Comput Biol*. 2010; 6. (f) Russ AP, Lampel S. The druggable genome: an update. *Drug Discov Today*. 2005; 10:1607–1610. [PubMed: 16376820] (g) Villoutreix BOL, C M, Lagorce D, Laconde G, Sperandio O. A leap into the chemical space of Protein-Protein Interaction inhibitors. *Curr Pharm Des*. 2012

Figure 1. Structural features of the acetyl-lysine binding pocket of the bromodomain

(A) The three-dimensional structure of the first bromodomain of human BRD4 (BRD4-BD1) (pdb code 3mxf), illustrating key amino acid residues, and five bound water molecules located at the acetyl-lysine binding pocket. The side chains of these residues are color-coded by atom type. (B) Surface representation of the BRD4-BD1 that is colored according to amino acid sequence conservation over the entire human bromodomain family (green is more conserved, whereas white is not conserved). (C) Acetyl-lysine (left) and a small molecule bromodomain inhibitor, JQ1 (right) shown when bound in the acetyl-lysine binding pocket (pdb codes 3uvx and 3mxf, respectively). The ligands, as well as bound water molecules are depicted in colored spheres according to atom type (red, blue, green, yellow and white for oxygen, nitrogen, carbon, sulfur, and hydrogen, respectively). The ligand binding site in the bromodomain protein is defined by mesh.